

Update on laboratory tests for the diagnosis and differentiation of hereditary angioedema and acquired angioedema

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ABSTRACT

The importance of laboratory testing in the diagnosis of hereditary angioedema (HAE) has increased with the advent of new treatment options in recent years. It has been 50 years since HAE was linked to a decrease of C1INH (the inhibitor of complement enzyme, C1 esterase), a link that provided for the first laboratory test available for this disorder. HAE is subdivided into types that can be differentiated only by laboratory testing. The Type I form is characterized by low levels and function of C1INH in the circulation. The Type II form is characterized by normal levels of C1INH, but low function. Sample collection and handling is critical for the functional assays. The serum samples for the functional analysis must be collected, separated, and frozen at less than -60°C within 2 hours of the blood draw. Additionally some suspected Type II patients may benefit from looking closely at what method is used for the functional testing. The acquired forms of angioedema (AAE) can benefit from the same clinical testing, because most are ultimately due to decreased C1INH. Measurement of C1q levels and testing for anti-C1INH autoantibodies can help differentiate AAE from HAE. Diagnostic testing for the third hereditary form, alternately called estrogen-dependent HAE, HAE with Normal C1INH or HAE Type III, still presents challenges, and definitive testing may have to wait until there is a more complete understanding of this mixed group of patients. The next steps will include genetic analysis of C1INH and other proteins involved in HAE.

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Hereditary angioedema (HAE) was first described over a 100 years ago, although the etiology of this disease was not established until the 1960s when it was discovered that partial deficiency of C1-inhibitor (C1INH) was involved.^{1–3} The molecule C1INH was initially described as “inhibitor of serum globulin permeability factor” for its role in the coagulation pathway, its most important role in the current discussion. Subsequently, C1INH was named for its role in the control of the classic pathway of complement.^{2–4} Even with the discovery of the involvement of C1INH, treatment options were largely limited to nonspecific treatments such as the use of androgens (see the accompanying overview articles for a full understanding of the role of C1INH in HAE and treatment options).^{3,4} More recently, the landscape of therapeutic options for HAE has changed dramatically in the United States with the release of three new treatment products and two more are awaiting approval. The advent of these new treat-

ments increases the importance of proper diagnosis and, in turn, the importance of quality laboratory testing. Several factors that impact laboratory results, and ultimately a diagnosis, are the subject of this article.

LABORATORY TESTS FOR THE DIAGNOSIS OF HAE

Three types of initial testing that are recommended to help in the diagnosis of most cases of HAE are C4 level, C1INH level, and C1INH function.^{5–7} Table 1 outlines our full recommendation of laboratory tests for diagnosis of HAE or acquired angioedema (AAE).^{8,19,20} C4 level is a common first line of testing because C4 can be measured with nephelometry in most clinical laboratories. Although a low level of C4 is indicative of HAE, it is not conclusive. Furthermore, there is mounting evidence that a normal C4 level may not be sufficient to exclude the diagnosis of HAE.⁹

Testing beyond C4 level allows for the differentiation between the types of HAE. HAE is divided into two diagnostic categories, type I and type II, which are similar in clinical presentation but vary in their genetic cause and, consequently, in their laboratory characteristics (see the accompanying article for further clinical description of the types of HAE).⁸ Type 1 patients show low levels of C1INH, resulting from any of a number of variant polymorphisms, all of which block C1INH production or secretion, resulting in low C1INH antigen levels in circulation. In contrast, the

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Table 1 Laboratory tests available for angioedema patients

Clinical Presentation		C4 Level	C1INH Function by Chromogenic Assay	C1INH Level	C1q Level	C1INH Auto-Antibody	Mutation in Factor XII Gene	Other tests that may be useful in special cases
Hereditary Angioedema (HAE)		Inherited defect in the gene for C1-esterase Inhibitor. Autosomal dominant: patients have one normal and one abnormal gene						
Type I	Recurrent episodic angioedema and abdominal attacks without urticaria. Onset in childhood or young adulthood. 75% have family history. Note that attacks may be estrogen dependent in this group as well as the Type III form.	Usually low		Normal or high	Normal	No	No	C2 low, C4a high, C4d:C4 ratio high
Type II		Usually low						
Type III		Normal						
Acquired Angioedema (AAE)		Condition not inherited but because of other underlying disease; C1-esterase Inhibitor is low because of consumption						
Type I	Onset age variable; symptoms same as HAE. No family history, may be associated with underlying lymphoproliferative disease.	Usually low		Low	Usually low may be normal	Usually No	NO	C3 may be low
Type II	Onset at variable age; symptoms indistinguishable from HAE. No family history, often associated with an autoantibody to C1INH.			Normal		Yes		

Most angioedema patients can be diagnosed from the clinical presentation supported by the laboratory tests listed in the table in the light grey boxes. Some patients may require further workup as suggested by the tests listed in the dark grey boxes.

C1INH = C1-inhibitor.

type II patient exhibits normal or even high levels of the C1INH protein in the circulation, but this protein is dysfunctional. Therefore, type II diagnosis requires functional testing of C1INH. The testing for the level of the C1INH antigen is relatively straightforward, but testing for functionality of the protein can be more problematic and therefore will be discussed in more detail.^{9,10}

CURRENT C1INH FUNCTION TESTS: OPTIONS TO CONSIDER

Current testing for C1INH function falls into two categories, an Enzyme Immuno Assay (EIA; Quidel, San Diego, CA); the second uses a chromogenic substrate (Technochrome; Technoclone GmbH, Vienna Austria; Berichrom, distributed by Siemens, Deerfield, IL). It is this second category that we use in our laboratory. Both types of tests measure function as a percentage of that from a normal serum. To understand the relative merits and differences of these two methods, it is necessary to understand the mechanism of action of C1INH.

C1INH is called a bait suicide inhibitor because it has an active loop that mimics the target sequence of the serine protease's substrate.⁷ The enzyme cleaves C1INH, but unlike the normal substrate, C1INH undergoes a conformational change that allows it to bind to the target enzyme, thus blocking further action of the enzyme and leading to clearance of the resulting enzyme/inhibitor complex from the circulation. This removal of the enzyme/inhibitor complex further reduces the level of circulating C1INH. The EIA-based assay measures the formation of the inhibitor/enzyme complex, specifically the formation of the C1INH/C1s complex. Although this is a prerequisite of inhibitor function, it is not a direct measure of inhibition.¹¹ The second method involves a chromogenic substrate that mimics C2, a natural substrate of C1s. When the chromogenic substrate is cleaved it yields a color that can be measured in the laboratory. The amount of color produced is inversely proportional to the functionality of the patient's C1INH.

In an evaluation conducted by Tarzi *et al.*, the reference ranges supplied by the manufacturer (<68% of normal) for the EIA tests were found to have a sensitivity of just 57%.⁹ In that study, the sensitivity increased to 78% when the range considered the lower limit of normal was adjusted (<84% of normal). Such an increase in sensitivity based on the laboratory-developed range rather than the package insert further points to the importance of choosing a laboratory that specializes in such testing and does the work to appropriately validate its own range.⁹

In 2008, Wagenaar-Bos and colleagues evaluated the effectiveness of C1INH function testing across labora-

tories and between methodologies. Blinded samples from normal subjects and from HAE patients were sent to 15 laboratories across Europe that specialize in HAE testing.¹⁰ Measurements of the same samples in different laboratories showed that there were no significant differences in the results between the two chromogenic assays available in Europe (listed previously). The authors caution against drawing conclusions regarding the EIA methodology because only two of 15 laboratories in their study used the EIA test. In this study, the chromogenic assays had a 98% positive and 100% negative predictive value, and the EIA-based method had a value of 100% and 62%, respectively. Similar to the aforementioned study the authors suggest that the high false negative rate for the EIA could be avoided with adjustment of the reference values away from the range suggested by the manufacturer.^{9,10}

In addition to the consideration of testing method, it is also important to consider sample handling and integrity when testing C1INH function. C1INH function is sensitive to sample handling, storage conditions, freezing, and thawing. The Complement Laboratory Division of the Advanced Diagnostic Laboratories (ADx) at National Jewish Health recommends collecting plasma with EDTA because it will help block *ex vivo* activation of complement or coagulation enzymes that may lead to an artificially low measurement of C1INH function that is not representative of what is actually happening in the patient.⁵ Additional recommendations include moving samples to storage on dry ice or to a freezer capable of temperatures below -60°C within 2 hours after the blood is drawn and the plasma is transferred to a fresh tube. A recent analysis by the ADx Complement Laboratory found that storage at -20°C for as little as 4 hours can lead to a decrease in C1INH function of 28% on average (Ashley Frazer-Abel, unpublished data, October 2010), similar to the data reported by Wagenaar-Bos.¹⁰ Therefore, properly freezing samples within 2 hours of collection can help avoid inconsistencies, particularly when C1INH values are thought to be at the low range of normal or borderline abnormal.^{5,10}

ADDITIONAL TESTING FOR HAE DIAGNOSIS

The role of C1INH in the control of complement allows testing of classic pathway complement activation to aid in diagnosis (see Table 1). Because the commonly available C4 quantitative measurement can not differentiate between intact C4 and C4 that has been cleaved by complement activation, some experts recommend measuring the C4:C4d ratio.¹¹ C4d is only produced when C4 cleavage (activation) has occurred. Therefore, the addition of the C4d measurement quantifies the level of ongoing activation rather than just the total level of C4, which is highly variable from person to

person. Other tests for complement activation could include C4a or C4 function measurements. Unfortunately, these assays are available only at highly specialized laboratories. In addition, testing of the full length C1INH gene, known as SERPING1, is now available, for C1INH, for diagnosis of types I and II at the genetic level.¹²

TYPE III HAE

A third form of HAE has recently been described.¹³ The new form has been given a number of names including estrogen-dependent angioedema, HAE with normal C1INH, and HAE type III. Although the third label had previously been used to describe a different set of patients, its brevity seems to have an appeal. As the first moniker indicates, this form is characteristically associated with increased estrogen levels and occurs predominately in women, although there are documented cases in men. As the second proposed name indicates, this form is differentiated from types I and II by having normal C1INH level *and* function. Bork and colleagues in Germany have studied a cohort of families in which affected members have one of two identified single nucleotide polymorphisms (SNPs) in the factor XII enzyme of the contact/coagulation pathway (Fig. 1).^{13,14} Currently, the genetic mapping of these defined SNPs is the only laboratory testing available to define this form of HAE. The ADx Complement Laboratory, in collaboration with the ADx Molecular Diagnostic Laboratory, is testing for these SNPs but still has to find either of them in the 40 individuals with type III phenotype from the local American population. We have found a few other polymorphisms in the

wider region of the gene but it is unknown what, if any, phenotypic consequence these polymorphisms would have for the protein. (Paul Reynolds, unpublished data, June 2011).⁵ Even in Bork's cohort, only ~30% of the affected population carried the SNPs.¹³ There is clearly considerable work remaining to be done to define the etiology of this form of HAE to move it from a diagnosis of exclusion.

DIFFERENTIATION OF AAE

An important step in the diagnosis of HAE includes ruling out the acquired forms of angioedema (AAE). As the name indicates, AAE is not associated with a family history (Table 1). Although this is helpful in distinguishing AAE from HAE, it is important to note that in some reports as many as 25% of HAE cases represent a spontaneous mutation that would not have been carried by preceding generations but may be passed to future ones.¹⁵

AAE is divided into two types. In both forms of AAE active complement consumption will generally result in a low C1q level; so the measurement of C1q can be a critical step in AAE diagnosis. Type I AAE is typically associated with an underlying lymphoproliferative disorder. Type II AAE is thought to be caused by the development of an autoantibody to the C1INH molecules.¹⁶ Such autoantibodies block the function of the inhibitor and also act as immune complexes that can activate complement and further deplete C1INH. Detection of the autoantibodies is difficult and possible at only a small number of highly specialized laboratories.

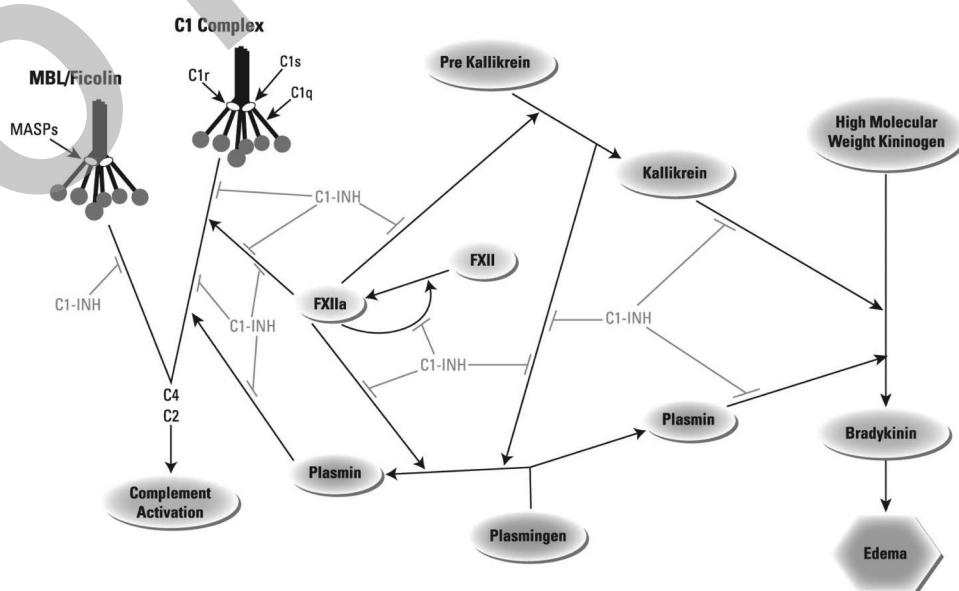


Figure 1. Targets of C1-inhibitor (C1INH) control in angioedema. Although C1INH was named for its role in controlling C1 of the complement pathway, it also has a role in controlling the contact/coagulation pathway. Control of the complement pathways is depicted to the left and the coagulation cascade to the right. A decrease in C1INH either by deficiency or by consumption will reduce the control of all the depicted serine proteases.

The process of HAE diagnosis can appear straightforward on paper, but because of the many reasons described here it can be much more complex in practice. Laboratory diagnosis of HAE is complicated by the fact that the patients are, with exceedingly rare exceptions, heterozygous for the variant C1INH gene so some level of normal C1INH can be measured.^{17,18}

This, added to the temperature sensitivity of complement in general and C1INH function in specific, further highlights the importance of care in sample collection and shipment as well as in choosing a laboratory that specializes in HAE and/or complement measurement. To control for such factors, it is recommended that testing be conducted twice on two separate blood draws to confirm the initial results of any suspected HAE patient. Additionally, interlaboratory variations in the type of tests and reference ranges should be considered when interpreting results. Regardless, categorizing these patients accurately can be difficult. These factors, in combination with the infrequency with which the patients may be encountered, points to the benefits of the physician and specialized laboratories working as a team.

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